APPENDIX:

The Appendix includes the following items:

☒ - a Declaration

☒ - Exhibits 1-6

PATENT

0546-1082

IN THE U.S. PATENT AND TRADEMARK OFFICE

In re application of

Steve HEALD et al. Conf. 4057

Application No. 10/550,945 Group 1651

Filed November 30, 2006 Examiner Irene Marx

PREPARATION OF VANILLIN FROM MICROBIAL TRANSFORMATION MEDIA BY EXTRACTION BY MEANS SUPERCRITICAL FLUIDS OR GASES

DECLARATION

Assistant Commissioner for Patents

P.O. Box 1450

Alexandria, VA 22313-1450

Sir:

1. I, **Mylène DARRICAU**, am a French citizen. I am employed by the French firm SAFISIS (a subsidiary of LESAFFRE), in the capacity of R&D Project Manager, and have held that position since January 5th, 2009. My background is a Master of Engineering in Biotechnology with 7 years of experience in Fermentation and Microbiology; I have a diploma from Engineering School of INSA in Toulouse (France), and I am in charge of Research and Development projects for SAFISIS in Biomolecules.

   I, **Evelyne FONCHY-PENOT**, hereby declare that I am a French citizen. I am employed by the French firm LESAFFRE INTERNATIONAL (a subsidiary of LESAFFRE), in the capacity of R&D Project Manager, and have held that position since 2000. LESAFFRE INTERNATIONAL is a service company which supports the different subsidiaries of LESAFFRE particularly in Research projects.

My background is PhD in biotechnology with more than 10 years experience in fermentation and Microbiology; I have a diploma from Engineer School from ESIL in Marseille (France), and I am in charge of Research and Development projects for LESAFFRE INTERNATIONAL in Biomolecules and Bioprocess.

2. We are familiar with the above-identified U.S. patent application, its prosecution before the United States Patent and Trademark Office, and the applied references of Rabenhorst et al. (U.S. Patent No. 6,133,003), Muheim et al. (U.S. Patent No. 6,235,507) and Makin (U.S. Patent No. 4,474,994).

3. In order to demonstrate the patentability of the present invention, we are submitting the following observations.

We confirm hereby that we have conducted the following experiments.

We state that, since my company got reports from independent laboratories that compare DSM 9991 and DSM 9992 of H&R patent and conclude that these two strains cannot be distinguished from one another; experiments were conducted on the sole DSM 9992 strain, exactly as disclosed in the H&R patent Examples 1 and 3.

We, Mrs DARRICAU and Mrs FONCHY-PENOT, who are researchers at SAF-ISIS and LESAFFRE INTERNATIONAL respectively, have realized the works detailed in the enclosed report (Exhibit 1)

As a summary the conclusions are that:

Strains DSM 9992 from US Patent No. 6,133,003 and Zyl 926 from the SAF-ISIS present application do not react at all the same way when applying, the same process as disclosed in examples 1 and 3 of US Patent No. 6,133,003.

Moreover, the HPLC analysis controls show that the ferulic acid was accumulated in the wort so that no bioconversion occurred with any of these two strains.

This means that the process in the US Patent No. 6,133,003is inoperatively disclosed in order to get the alleged results regarding vanillin production.

As a consequence we state that the prior art was duly worked out with the intention to reduce to practice the working examples of US Patent No. 6,133,003, with the above mentioned absence of results.

We state that we made all reasonable due efforts, that a person of ordinary skill in the art should make, by taking all the information given in the specification such as for instance pH and oxygen maintenance, and also by using our general knowledge for example, to control the foaming that occurred during the fermentation by adding an antifoam.

Accordingly, we consider that, contrary to the DSM 9991 and 9992 strains of US Patent No. 6,133,003, the Zyl 926 microorganism, of the present application of SAF-ISIS, deposited within CABI as IMI 390106 for the purposes of patent procedures under the Budapest Treaty, provides unexpected results.

The latter are demonstrated notably by:

-a consumption of ferulic acid with concomitant production of above 11 g/L of vanillin

-a vanillin product obtained there from absent of odoriferous by-products and with none of guaïacol, vinyl guaïacol, eugenol and isoeugenol being present at more than 100ppm.

4. We declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date June 29, 2010

Mylène DARRICAU
R&D Product Manager
SAF-ISIS
Zone Artisanale
Soustons 40140 – France

Evelyne FONCHY-PENOT
R&D Project Manager
LESAFFRE INTERNATIONNAL
Rue Gabriel Peri 147
Marcq en Baroeul 59700 – France

Exhibit 1

Report on the "Characterisation of Strains belonging to the Genus *Amycolatopsis* by 16S rRNA Gene Sequencing"

Professor M. Goodfellow

Background

Initially, the client wished to have the taxononic relationships between the three *Amycolatopsis* strains determined by 16S rRNA gene sequencing. This study was designed to establish the inferred phylogenetic relationships between the cultures and their position (s) in the *Amycolatopsis* 16S rRNA gene tree.

Receipt of Strains

The package containing the strains arrived intact on May 20th (See photographs attached).

Materials and Methods

Cultivation of strains. The strains were grown in shake flasks (150 rpm) in ISP 2 broth for 48 hours at 37°C. Following incubation, biomass was harvested by centrifugation.

16S rRNA gene sequencing analyses. Chromosomal DNA was isolated and 16S rRNA PCR was done using well established procedure (Lane, 1991). PCR product was purified and direct sequencing of 16S rRNA genes of the three *Amycolatopsis* strains was carried out using BigDyeTM version 3.1 Terminator Cycle Sequencing Kit (PE Applied Biosystems, MA). Samples were completed using 10 ng of DNA for every 100 bp of template DNA and 3.2 pmol of primer. Post-sequencing reactions were purified away from unincorporated dye terminators using Performa DTR spin-columns (EdgeBiosystems, MD). Sequences were determined using an ABI 3730 Sequencer. The resultant almost complete 16S rRNA gene sequences (1360 nucleotides) were aligned manually against corresponding sequences of the type strains of *Amycolatopsis* species, retrieved from GenBank, using PHYDIT software (Chun, 1995). A phylogenetic tree was inferred by using neighbour-joining (Saitou & Nei, 1987) tree-making algorithm from the PHYDIT program and an evolutionary distance matrix generated by using the distance model of Jukes & Cantor (1969). The robustness of the phyletic lines recovered in the *Amycolatopsis* tree were determined in a bootstrap analysis (Felsenstein, 1985) of the neighbour-joining dataset by using the SEQBOOT and CONSENSE options from the PHYLIP suite of programs (Felsenstein, 1993).

Results

It is apparent from Figures 1 to 3 that the 16S rRNA gene sequences obtained for the three *Amycolatopsis* strains are of high quality as there are no ambiguities in the conserved regions of the traces. It is clear from the 16S rRNA *Amycolatopsis* tree that the three strains belong to the *Amycolatopsis methanolica* subclade, the taxononic integrity of the latter is underpinned by a 100% bootstrap value (Fig. 4). It is also apparent from the tree and from the associated nucleotide similarity and difference

matrix (Table 1) that the three strains share identical 16S rRNA gene sequences with one another.

Conclusions

- The three *Amycolatopsis* strains belong to the *Amycolatopsis methanolica* subclade in the 16S rRNA *Amycolatopsis* gene tree.

- The three *Amycolatopsis* strains shared identical 16S rRNA gene sequences with one another.

References

Chun, J. (1995). Computer-assisted classification and identification of actinomycetes. Ph.D. Thesis, University of Newcastle, Newcastle upon Tyne, UK.

Felsenstein, J. (1985). Confidence limits on phylogeny: An appropriate use of the bootstrap. *Evolution* **39**, 7783-7791.

Felsenstein, J. (1993). PHYLIP- Phylogenetic Inference Package version 3.5.1. Department of Genetics, University of Washington, Seattle, USA.

Jukes, T.H. & Cantor, C.R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, volume **3**, pp. 21-132. Edited by H. N. Munro, Academic Press, New York.

Lane, D.J. (1991). 16S/23S rRNA Sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115-148. Edited by E. Stackebrandt & M. Goodfellow. John Wiley & Sons, Chichester, New York.

Saitou, N. & Nei, M. (1987). The neighbour-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**, 406-425.

Attachments with the Report

Legends for Figures.

Figs. 1a-3b. Chromatograms of Samples A, B and C respectively (a- sequencing with forward primer; b- sequencing with reverse primer).

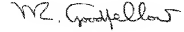
Fig. 4. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing relationships between the three *Amycolatopsis* strains and between them and the type strains of *Amycolatopsis* species. Numbers at the nodes indicate the level of bootstrap support (%) based on neighbour-joining analysis of 1000 resampled datasets; only values above 50% are given. The scale bar indicates 0.02 substitutions per nucleotide position.

Photographs.

Photographs 1-5. Receipt of strains.

Tables.

Table 1. Nucleotide similarity and difference matrix with the three strains which share identical 16S rRNA gene sequences with one another.



Professor Michael Goodfellow

Exhibit 2

CCFRA Technology Limited
Station Road, Chipping Campden
Gloucestershire
GL55 6LD, United Kingdom

Tel: +44 (0)1386 842000
Fax: +44 (0)1386 842100
www.campden.co.uk

Campden BRI
food and drink innovation

Richard Tallon
LeSaffre Safisis
Zone Artisanale
40140 Soustons
France

Report No: MB/REP/MMR/115732/00002-00004/2
Report Date: 30/06/09

CONFIDENTIAL CERTIFICATE OF ANALYSIS

Microbiology Department
MicroID

Notes

The results provided herein relate only to the items tested.

RiboPrint®, RiboGroup® and RiboPrinter™ are trademarks of Qualicon™ L.L.C., a subsidiary of E.I. DuPont de Nemours and Company, Wilmington, Delaware, USA.

Prepared/Checked By:

Authorised By:

Authorised Signatories:	C. Mitchell Research Technical Officer	
	S. Jordan Molecular Methods Manager	
	C. Baylis Methods Research Manager	✓

Any opinions and interpretations contained in this report are outside the scope of UKAS accreditation.

This test report or certificate shall not be reproduced, except in full, without written approval of Campden BRI.

Confidentiality notice: The information in this document is only intended for the individual or entity to whom it is addressed. It may contain privileged and confidential information that is exempt from disclosure by law and if you are not the intended recipient, you must not copy, distribute or take any action in reliance on it. If you have received this document in error, please notify Campden BRI immediately by telephone.

CCFRA Technology Limited, Registered No. 2836922, Incorporated in England & Wales
Registered Office: Station Road, Chipping Campden, Glos. GL55 6LD
Part of Campden BRI Group

Information emanating from Campden BRI is given after the exercise of all reasonable care and skill in its compilation, preparation and issue, but is provided without liability in its application and use.

Summary

Three bacterial isolates were sent to Campden BRI from SAFISIS for strain comparison using the Qualicon™ RiboPrinter® at the request of the client. The parcel was received on site unopened as shown in the images taken of the package in Appendix 1.

The isolates were stored and grown prior to analysis as detailed in the NDA supplied by the client. Standard techniques and reagents were used for the ribotyping. The enzyme PvuII was used for the analysis of the isolates as it is has been shown to differentiate between DuPont database entries for *Streptomyces* sp. The results of the work are noted in Tables below, with a pattern comparison shown in Figure 1. A summary of the RiboPrinter methodology is outlined in Appendix 2.

CCFRA Code:	MMR/115732/00002	**Date of Receipt:**	20/05/09
Customer Code	Sample A	**Client presumptive identification**	*Amycolatopsis* sp
Sample Description:	Frozen culture		
Comments:	Stored at ambient temperature prior to analysis		

Analysis Type	Test Details	Identification	Comments
RiboPrinter®	*Pvu*II	None	Isolate fell outside of 0.85 similarity needed for automatic identification against the DuPont identification database. The isolate was placed in RiboGroup 114-3210-S-1.
RiboPrinter®	Strain comparison		Refer to Figure 1 for details

CCFRA Code:	MMR/115732/00003	**Date of Receipt:**	20/05/09
Customer Code	Sample B	**Client presumptive identification**	*Amycolatopsis* sp
Sample Description:	Freeze Dried Ampoule		
Comments:	Stored at ambient temperature prior to analysis		

Analysis Type	Test Details	Identification	Comments
RiboPrinter®	*Pvu*II	None	Isolate fell outside of 0.85 similarity needed for automatic identification against the DuPont identification database. The isolate was placed in RiboGroup 114-3210-S-1.
RiboPrinter®	Strain comparison		Refer to Figure 1 for details

CCFRA Code:	MMR/115732/00004	**Date of Receipt:**	20/05/09
Customer Code	Sample C	**Client presumptive identification**	*Amycolatopsis* sp
Sample Description:	Freeze Dried Ampoule		
Comments:	Stored at ambient temperature prior to analysis		

Analysis Type	Test Details	Identification	Comments
RiboPrinter®	*Pvu*II	None	Isolate fell outside of 0.85 similarity needed for automatic identification against the DuPont identification database. The isolate was placed in RiboGroup 114-3210-S-1.
RiboPrinter®	Strain comparison		Refer to Figure 1 for details

Strain comparison

A comparison of the patterns generated for the three isolates is shown below in Figure 1a and b.

Figure 1a: Strain comparison of isolates MB/115732/2-4 using MB/115732/2 as the reference isolate

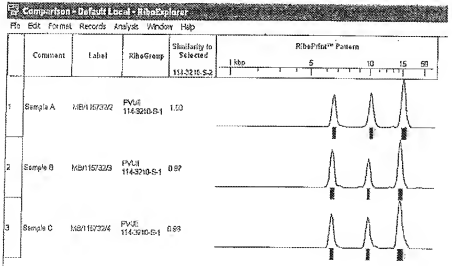
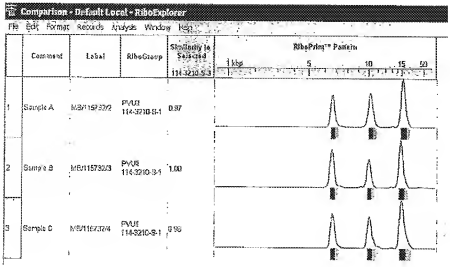


Figure 1b: Strain comparison of isolates MB/115732/2-4 using MB/115732/3 as the reference isolate

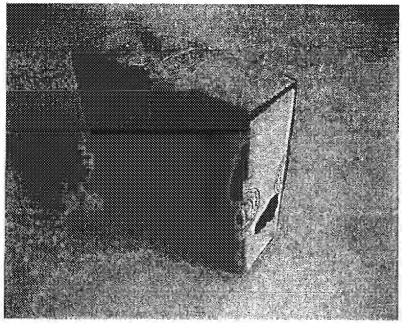


All three isolates were placed in the same Ribogroup 114-3210-S-1. These results suggest that the isolates are indistinguishable from each other using PvuII.

These data readily demonstrate the ability of the RiboPrinter system to characterise and discriminate bacterial strains by analysis of RiboPrint patterns. This information will be available for reference on the archived database and as such can be used to authenticate, or compare to, these cultures time and time again.

These isolates will be kept for a standard period of 4 weeks after ribotyping after which they will be destroyed as outlined in the NDA supplied by the client.

Appendix 1: Image of package received for analysis at Campden BRI



The RiboPrinter system is an automated ribotyping process as follows:

Bacterial cells are broken open to release the genomic DNA into solution. This DNA is digested ('cut up') using a restriction enzyme to generate fragments of different lengths, which are then separated out on the basis of size. Ribosomal DNA is detected using probes which give off light that is captured by a camera. The resulting image is converted into a RiboPrint pattern that can be characterised (into RiboGroups) and where possible identified by proprietary software. RiboGroups are assigned to patterns in the following format - RiboGroup library - Machine number - batch number and sample position where the pattern occurred for the first time for example RIBO1 114-1002-S-1

As each sample is analysed by the software, it makes a decision about how closely the new sample pattern matches the existing patterns in the database. If the software finds a match, that is if it is unable to distinguish the new pattern from an existing pattern (i.e. >92% similarity between patterns), it assigns the new sample to that RiboGroup. All samples in a RiboGroup have the same RiboGroup code. Thus if the isolate has been previously ribotyped on this instrument, the instrument database will recognise the reproduced RiboPrint and allocate the RiboGroup code assigned in the first instance.

When an isolate is analysed on the machine for the first time, i.e. it does not match with any existing patterns, it will receive a new code i.e. the batch and sample number specific to that new batch run.

For automatic (DuPont) identification, the new sample information is compared to existing patterns within a supplied (DUP) database. If the software finds a match of >85% a DuPont identification will be reported.

If no automatic identification is given, the user can instruct the software to show the percentage similarities between existing patterns in the instrument sample database (Campden BRI database) and the new sample pattern. When >85% similarity is shown identification can be offered.

Exhibit 3

PHYDIT - Similarity analysis
1360 nucleotides analysed
Lower-left triangle contains [NT] Similarity.
Uppper-right triangle contains [NT] Different/Total nucleotides.

The following table contains tab-delimited numbers. Copy and paste to MS Excel or Word.

	Amycolatopsis Sample A
Amycolatopsis Sample A	---
Amycolatopsis Sample B	100
Amycolatopsis Sample C	100
Amycolatopsis methanolica DSM 44096T (AJ249135)	98.88
Amycolatopsis thermoflava DSM 44574T (AF052390)	98.88
Amycolatopsis jejuensis NRRL B-24427T (DQ000200)	94.25
Amycolatopsis sulphurea DSM 46092T (AJ293756)	95.37
Amycolatopsis alba DSM 44262T (AF051340)	94.76
Amycolatopsis coloradensis DSM 44225T (AJ293753)	94.47
Amycolatopsis regifaucium DSM 45072T (AY129760)	94.84
Amycolatopsis azurea DSM 43854T (AJ400709)	94.69
Amycolatopsis orientalis DSM 40040T (AJ400711)	94.69
Amycolatopsis keratiniphila subsp. keratiniphila DSM 4440T (AJ278496)	94.69
Amycolatopsis keratiniphila subsp. nogabecina DSM 445T (AJ508238)	94.69
Amycolatopsis japonica DSM 44213T (AJ508236)	94.54
Amycolatopsis decaplanina DSM 44594T (AJ508237)	94.77
Amycolatopsis minnesotensis NRRL B-24435 (DQ076482)	94.84
Amycolatopsis nigrescens DSM 44992T (DQ486888)	95.44
Amycolatopsis lexingtonensis DSM 44653T (AY183358)	94.61
Amycolatopsis pretoriensis DSM 44654T (AY183356)	94.54
Amycolatopsis rifamycinica DSM 46095T (AY083603)	94.69
Amycolatopsis kentuckyensis DSM 44652T (AY183357)	94.54
Amycolatopsis balhimycina DSM 44591T (AJ508239)	94.47
Amycolatopsis vancoresmycina DSM 44592T (AJ508240)	94.84
Amycolatopsis plumensis DSM 44776T (AY262825)	94.61
Amycolatopsis tolypomycina DSM 44544T (AJ293757)	94.61
Amycolatopsis mediterranei DSM 43304T (AJ293754)	94.84
Amycolatopsis australiensis DSM 44671T (AY129753)	95.14
Amycolatopsis saalfeldensis DSM 44993T (DQ792500)	95.07
Amycolatopsis echigonensis JCM 21831T (AB248535)	94.84
Amycolatopsis niigatensis JCM 21832T (AB248537)	95.06
Amycolatopsis benzoatilytica DSM 43387T (AY957506)	93.39
Amycolatopsis albidoflavus DSM 44639T (AJ252832)	94.9
Amycolatopsis halotolerans NRRL B-24428T (DQ000196)	95.21
Amycolatopsis rubida DSM44637T (AF222022)	94.51
Amycolatopsis sacchari DSM 44468T (AF223354)	95.29
Amycolatopsis taiwanensis NBRC 102103T (DQ160215)	94.99
Amycolatopsis marina NBRC 104263T (EU329845)	95.96
Amycolatopsis palatopharyngis JCM 12460T (AF479268)	96.19
Amycolatopsis fastidiosa DSM 43855T (AB184566)	92.38

Amycolatopsis Sample B	Amycolatopsis Sample C
0/1338	0/1338
---	0/1338
100	---
98.88	98.88
98.88	98.88
94.25	94.25
95.37	95.37
94.76	94.76
94.47	94.47
94.84	94.84
94.69	94.69
94.69	94.69
94.69	94.69
94.69	94.69
94.54	94.54
94.77	94.77
94.84	94.84
95.44	95.44
94.61	94.61
94.54	94.54
94.69	94.69
94.54	94.54
94.47	94.47
94.84	94.84
94.61	94.61
94.61	94.61
94.84	94.84
95.14	95.14
95.07	95.07
94.84	94.84
95.06	95.06
93.39	93.39
94.9	94.9
95.21	95.21
94.51	94.51
95.29	95.29
94.99	94.99
95.96	95.96
96.19	96.19
92.38	92.38

Amycolatopsis methanolica DSM 44096T (AJ249135)
15/1338
15/1338
15/1338

99.85
94.32
94.99
94.69
94.47
94.39
94.62
94.54
94.77
94.61
94.47
94.92
94.54
95.74
94.61
94.54
94.62
94.47
94.39
94.47
94.17
94.24
94.76
94.99
95.22
94.54
94.76
92.93
94.75
94.91
94.81
95.74
95.44
96.34
96.49
92.38

Amycolatopsis thermoflava DSM 44574T (AF052390)
15/1337
15/1337
15/1337
2/1337

94.24
94.99
94.61
94.39
94.31
94.54
94.47
94.69
94.54
94.39
94.84
94.47
95.66
94.54
94.47
94.54
94.39
94.32
94.39
94.09
94.16
94.68
94.91
95.14
94.46
94.68
92.85
94.67
94.83
94.73
95.66
95.36
96.26
96.41
92.3

Amycolatopsis jejuensis NRRL B-24427T (DQ000200)	
77/1338	97.83
77/1338	96.86
77/1338	96.64
76/1338	97.08
77/1337	97.01
---	96.79
	96.64
	96.86
	97.01
	96.79
	96.71
	96.71
	96.56
	96.64
	96.64
	96.41
	96.19
	96.64
	96.34
	96.78
	96.48
	97.01
	97.16
	97.23
	97.16
	95.29
	97
	96.86
	96.77
	96.64
	93.88
	95.44
	95.52
	90.44

Amycolatopsis sulphurea DSM 46092T (AJ293756)		Amycolatopsis alba DSM 44262T (AF051340)	
62/1338		70/1337	
62/1338		70/1337	
62/1338		70/1337	
67/1338		71/1337	
67/1337		72/1336	
29/1339		42/1337	
---		45/1337	
	96.63	---	
	96.49		99.25
	97.38		98.28
	97.01		98.65
	96.94		98.58
	96.79		98.43
	97.01		98.5
	96.94		98.28
	97.31		98.35
	97.01		97.38
	97.09		97.53
	96.63		97.83
	96.56		97.91
	96.56		97.98
	96.34		97.75
	96.26		97.68
	96.71		97.68
	96.11		97.16
	96.41		97.6
	96.41		97.9
	97.09		97.46
	97.16		97.83
	97.31		97.38
	97.68		97.53
	95.67		95.82
	97.68		97.6
	97.68		97.53
	96.92		97.22
	97.23		96.19
	93.58		94.09
	95.52		95.14
	95.67		95.44
	91.04		91.1

Amycolatopsis coloradensis DSM 44225T (AJ293753)
74/1338
74/1338
74/1338
74/1338
75/1337
45/1338
47/1338
10/1337

98.5
98.58
98.8
98.65
98.65
98.65
98.58
97.61
97.38
97.83
97.91
97.83
97.61
97.68
97.46
97.08
97.53
97.68
97.46
97.91
97.08
97.23
95.59
97.45
97.23
97.14
95.89
93.87
95.07
95.37
90.81

Amycolatopsis regifaucium DSM 45072T (AY129760)
69/1337
69/1337
69/1337
75/1337
76/1336
39/1337
35/1337
23/1336
20/1337

98.88
99.1
98.88
99.25
99.48
99.1
97.46
97.01
97.46
97.38
97.38
97.16
97.01
97.46
97.01
97.38
97.3
97.83
97.23
97.83
98.13
96.43
98.05
98.13
97.29
95.96
93.34
95.29
95.29
91.17

Amycolatopsis azurea DSM 43854T (AJ400709)	Amycolatopsis orientalis DSM 40040T (AJ400711)	
71/1338	71/1338	
71/1338	71/1338	
71/1338	71/1338	
72/1338	73/1338	
73/1337	74/1337	
40/1338	43/1338	
40/1338	41/1338	
18/1337	19/1337	
19/1338	16/1338	
15/1337	12/1337	
---	6/1338	
	99.55 ---	
	99.18	99.33
	99.48	99.63
	99.1	99.18
	99.18	99.33
	97.16	97.31
	97.31	97.31
	98.13	97.83
	98.06	97.76
	97.91	97.61
	97.68	97.38
	97.68	97.38
	97.53	97.38
	96.93	96.86
	97.46	97.38
	97.75	97.46
	97.76	97.76
	97.76	97.46
	97.61	97.38
	97.9	97.68
	96.43	96.35
	97.9	97.83
	97.75	97.68
	97.67	97.44
	96.26	96.26
	93.87	93.65
	95.74	95.59
	96.04	95.81
	91.63	91.63

Amycolatopsis keratiniphila subsp. keratiniphila DSM 44407T (AJ278496)

71/1338	99.7
71/1338	99.25
71/1338	99.33
70/1338	97.31
71/1337	97.53
45/1338	97.83
43/1338	97.76
21/1337	97.46
18/1338	97.23
15/1337	97.23
11/1338	97.46
9/1338	96.86
---	97.31
	97.38
	97.61
	97.38
	97.76
	97.9
	96.05
	97.98
	97.75
	97.89
	96.49
	93.87
	95.89
	96.11
	91.55

Amycolatopsis keratiniphila subsp. nogabecina DSM 445T (AJ508238)	
71/1337	99.33
71/1337	99.48
71/1337	97.31
72/1337	97.38
73/1336	97.75
42/1337	97.68
40/1337	97.53
20/1336	97.31
18/1337	97.31
10/1336	97.46
7/1337	96.78
5/1337	97.31
4/1337	97.38
---	97.76
	97.38
	97.83
	98.13
	96.43
	98.05
	97.98
	97.89
	96.41
	93.79
	95.74
	95.96
	91.77

Amycolatopsis japonica DSM 44213T (AJ508236)		Amycolatopsis decaplanina DSM 44594T (AJ508237)	
73/1338		70/1338	
73/1338		70/1338	
73/1338		70/1338	
74/1338		68/1338	
75/1337		69/1337	
40/1338		43/1338	
41/1338		36/1338	
23/1337		22/1337	
18/1338		19/1338	
7/1337		12/1337	
12/1338		11/1338	
11/1338		9/1338	
10/1338		9/1338	
9/1337		7/1337	
---		11/1338	
	99.18	---	
	97.23		97.09
	97.16		97.23
	97.83		97.38
	97.76		97.31
	97.61		97.31
	97.38		97.08
	97.23		96.94
	97.31		97.09
	96.93		96.48
	97.46		96.93
	97.46		97.31
	97.68		97.98
	97.38		97.09
	97.53		97.68
	97.75		98.13
	95.9		96.12
	97.75		97.98
	97.6		97.98
	97.37		97.67
	95.96		96.19
	93.57		93.57
	95.52		95.89
	95.59		95.81
	91.11		91.41

Amycolatopsis minnesotensis NRRL B-24435 (DQ076482)	
69/1338	98.43
69/1338	96.71
69/1338	96.79
73/1338	96.64
74/1337	96.41
44/1339	96.56
40/1339	97.38
35/1337	96.26
32/1338	96.56
34/1337	96.63
38/1338	96.94
36/1338	97.76
36/1338	96.41
36/1337	96.56
37/1338	95.14
39/1338	96.78
---	96.56
	96.24
	96.86
	93.13
	95.96
	96.11
	91.04

Amycolatopsis nigrescens DSM 44992T (DQ486888)	
61/1338	
61/1338	
61/1338	
57/1338	
58/1337	
44/1339	
39/1339	
33/1337	
35/1338	
40/1337	
36/1338	
36/1338	
33/1338	
35/1337	
38/1338	
37/1338	
21/1339	

	96.71
	96.79
	96.79
	96.56
	96.49
	96.64
	96.04
	96.41
	96.78
	97.09
	97.98
	96.49
	96.64
	94.84
	96.78
	96.64
	96.7
	97.53
	93.5
	96.64
	96.64
	91.78

Amycolatopsis lexingtonensis DSM 44653T (AY183358)	
72/1337	99.93
72/1337	99.63
72/1337	99.4
72/1337	99.25
73/1336	98.95
46/1337	98.8
45/1337	99.1
29/1336	99.1
29/1337	98.28
34/1336	98.5
25/1337	97.6
29/1337	97.75
29/1337	96.65
30/1336	97.97
29/1337	97.75
35/1337	97.59
44/1337	96.34
44/1337	94.32
---	95.36
	95.51
	91.47

Amycolatopsis pretoriensis DSM 44654T (AY183356)	
73/1338	99.7
73/1338	99.48
73/1338	99.33
73/1338	99.03
74/1337	98.88
45/1339	99.18
46/1339	99.18
28/1337	98.36
28/1338	98.58
35/1337	97.53
26/1338	97.68
30/1338	96.58
30/1338	97.9
31/1337	97.68
30/1338	97.52
36/1338	96.41
43/1339	94.4
43/1339	95.29
1/1337	95.44
---	91.41

Amycolatopsis rifamycinica DSM 46095T (AY083603)	
71/1338	99.63
71/1338	99.18
71/1338	99.03
72/1338	98.65
73/1337	98.95
45/1338	99.33
46/1338	98.36
27/1337	98.43
29/1338	97.68
35/1337	97.83
28/1338	96.43
32/1338	97.9
34/1338	97.83
33/1337	97.52
32/1338	96.34
36/1338	94.32
45/1338	95.37
43/1338	95.37
5/1337	91.41
4/1338	

Amycolatopsis kentuckyensis DSM 44652T (AY183357)
73/1337
73/1337
73/1337
74/1337
75/1336
48/1337
49/1337
30/1336
32/1337
38/1336
31/1337
35/1337
37/1337
36/1336
35/1337
39/1337
48/1337
46/1337
8/1336
7/1337
5/1337

99.1
98.95
98.58
98.88
99.25
98.28
98.2
97.46
97.6
96.2
97.67
97.75
97.44
96.04
94.02
95.21
95.21
91.1

Amycolatopsis balhimycina DSM 44591T (AJ508239)	
74/1338	98.65
74/1338	98.35
74/1338	98.65
75/1338	99.18
76/1337	98.28
51/1338	98.36
50/1338	97.16
31/1337	97.31
31/1338	96.2
40/1337	97.53
31/1338	97.46
35/1338	97.29
37/1338	95.96
36/1337	94.17
37/1338	94.92
41/1338	95.07
46/1338	91.11
47/1338	
10/1337	
9/1338	
11/1338	
12/1337	

Amycolatopsis vancoresmycina DSM 44592T (AJ508240)	
69/1338	98.65
69/1338	98.95
69/1338	98.65
74/1338	98.36
75/1337	98.13
45/1338	97.91
44/1338	98.05
31/1337	96.66
34/1338	98.13
34/1337	98.2
33/1338	97.59
35/1338	96.26
34/1338	94.17
34/1337	95.29
36/1338	95.29
39/1338	91.33
35/1338	
45/1338	
14/1337	
13/1338	
13/1338	
14/1337	
18/1338	

Amycolatopsis plumensis DSM 44776T (AY262825)
72/1337
72/1337
72/1337
78/1337
79/1336
49/1337
52/1337
38/1336
39/1337
40/1336
41/1337
42/1337
42/1337
43/1336
41/1337
47/1337
50/1337
53/1337
16/1336
15/1337
18/1337
19/1336
22/1337
18/1337
--

99.1
98.28
97.83
97.46
97.38
97.45
96.27
97.45
97.6
96.84
95.74
93.79
94.76
94.99
90.8

Amycolatopsis tolypomycina DSM 44544T (AJ293757)
72/1337
72/1337
72/1337
77/1337
78/1336
43/1337
48/1337
32/1336
33/1337
35/1336
34/1337
35/1337
36/1337
36/1336
34/1337
41/1337
46/1337
48/1337
12/1336
11/1337
14/1337
15/1336
18/1337
14/1337
12/1336

98.58
98.2
97.91
97.6
97.75
96.58
97.82
97.9
97.14
95.89
94.02
95.06
95.36
91.1

Amycolatopsis mediterranei DSM 43304T (AJ293754)	
69/1336	98.5
69/1336	98.35
69/1336	97.38
70/1336	97.53
71/1335	96.12
47/1336	97.6
48/1336	97.68
28/1335	97.36
31/1336	96.11
36/1335	94.01
30/1336	95.36
34/1336	95.36
35/1336	91.39
35/1335	
34/1336	
36/1336	
45/1336	
43/1336	
12/1335	
11/1336	
9/1336	
10/1335	
11/1336	
18/1336	
23/1335	
19/1335	

Amycolatopsis australiensis DSM 44671T (AY129753)	
65/1338	
65/1338	
65/1338	
67/1338	
68/1337	
40/1338	
39/1338	
34/1337	
34/1338	
29/1337	
30/1338	
30/1338	
32/1338	
30/1337	
31/1338	
27/1338	
41/1338	
39/1338	
23/1337	
22/1338	
22/1338	
23/1337	
23/1338	
22/1338	
29/1337	
24/1337	
20/1336	

	97.91
	97.46
	97.75
	96.35
	97.98
	97.9
	97.52
	96.71
	94.25
	95.96
	95.81
	91.41

Amycolatopsis saalfeldensis DSM 44993T (DQ792500)	
66/1338	
66/1338	
66/1338	
64/1338	
65/1337	
38/1338	
38/1338	
29/1337	
28/1338	
37/1337	
30/1338	
34/1338	
35/1338	
35/1337	
35/1338	
39/1338	
30/1338	
27/1338	
20/1337	
19/1338	
21/1338	
24/1337	
22/1338	
25/1338	
34/1337	
28/1337	
22/1336	
28/1338	

	97.16
	97.31
	95.74
	97.53
	97.31
	97.22
	97.01
	94.39
	95.74
	95.89
	91.48

Amycolatopsis echigonensis JCM 21831T (AB248535)
69/1337
69/1337
69/1337
73/1337
74/1336
37/1338
36/1338
35/1336
39/1337
29/1336
32/1337
35/1337
30/1337
29/1336
33/1337
31/1337
48/1338
47/1339
32/1336
33/1338
31/1337
34/1336
38/1337
28/1337
35/1336
32/1336
35/1335
34/1337
38/1337

99.4
97.19
98.95
99.1
98.5
96.19
94.02
95.44
95.29
90.88

Amycolatopsis niigatensis JCM 21832T (AB248537)
66/1336
66/1336
66/1336
70/1336
71/1335
38/1337
31/1337
33/1335
37/1336
25/1335
28/1336
31/1336
28/1336
25/1335
30/1336
25/1336
46/1337
45/1338
30/1335
31/1337
29/1336
32/1335
36/1336
26/1336
34/1335
30/1335
33/1334
30/1336
36/1336
8/1338

97.57
99.18
99.55
98.42
96.18
93.94
95.43
95.21
91.25

Amycolatopsis benzoatilytica DSM 43387T (AY957506)	
87/1316	
87/1316	
87/1316	
93/1316	
94/1315	
62/1316	
57/1316	
55/1315	
58/1316	
47/1315	
47/1316	
48/1316	
52/1316	
47/1315	
54/1316	
51/1316	
64/1316	
68/1317	
44/1315	
45/1316	
47/1316	
50/1315	
50/1316	
44/1316	
49/1315	
45/1315	
51/1314	
48/1316	
56/1316	
37/1316	
32/1316	

	97.41
	97.42
	96.64
	94.53
	92.17
	93.54
	93.62
	90.05

Amycolatopsis albidoflavus DSM 44639T (AJ252832)	
68/1334	
68/1334	
68/1334	
70/1334	
71/1333	
40/1335	
31/1335	
32/1333	
34/1334	
26/1333	
28/1334	
29/1334	
27/1334	
26/1333	
30/1334	
27/1334	
43/1335	
43/1336	
27/1333	
28/1335	
28/1334	
31/1333	
33/1334	
25/1334	
34/1333	
29/1333	
32/1332	
27/1334	
33/1334	
14/1336	
11/1336	
34/1314	

	99.18
	98.5
	96.33
	93.93
	95.35
	95.28
	91.32

Amycolatopsis halotolerans NRRL B-24428T (DQ000196)
64/1336
64/1336
64/1336
68/1336
69/1335
42/1337
31/1337
33/1335
37/1336
25/1335
30/1336
31/1336
30/1336
27/1335
32/1336
27/1336
46/1337
45/1338
30/1335
31/1337
29/1336
30/1335
34/1336
24/1336
32/1335
28/1335
31/1334
28/1336
36/1336
12/1338
6/1338
34/1316
11/1336

98.42
96.18
93.72
95.28
95.21
91.25

Amycolatopsis rubida DSM44637T (AF222022)	Amycolatopsis sacchari DSM 44468T (AF223354)	
73/1330	63/1338	
73/1330	63/1338	
73/1330	63/1338	
69/1330	57/1338	
70/1329	58/1337	
43/1331	45/1338	
41/1331	37/1338	
37/1329	51/1337	
38/1330	55/1338	
36/1329	54/1337	
31/1330	50/1338	
34/1330	50/1338	
28/1330	47/1338	
28/1329	48/1337	
35/1330	54/1338	
31/1330	51/1338	
50/1331	42/1338	
44/1332	33/1338	
32/1329	49/1337	
33/1331	48/1338	
33/1330	49/1338	
34/1330	53/1337	
36/1330	54/1338	
32/1330	50/1338	
42/1329	57/1337	
38/1329	55/1337	
35/1328	52/1336	
33/1330	44/1338	
37/1330	40/1338	
20/1332	51/1337	
21/1332	51/1336	
44/1310	72/1316	
20/1330	49/1334	
21/1332	51/1336	
---	46/1330	
	96.54 ---	
	93.99	94.77
	95.79	96.26
	95.64	96.41
	90.98	91.85

Amycolatopsis taiwanensis NBRC 102103T (DQ160215)
67/1338
67/1338
67/1338
61/1338
62/1337
82/1339
86/1339
79/1337
82/1336
89/1337
82/1338
85/1338
82/1338
83/1337
86/1338
86/1338
92/1339
87/1339
76/1337
75/1339
76/1338
80/1337
78/1338
78/1338
83/1337
80/1337
80/1336
77/1338
75/1338
80/1338
81/1337
103/1316
81/1335
84/1337
80/1331
70/1338

94.1
94.02
89.92

Amycolatopsis marina NBRC 104263T (EU329845)
54/1338
54/1338
54/1338
49/1338
50/1337
61/1338
60/1338
65/1337
66/1338
63/1337
57/1338
59/1338
55/1338
57/1337
60/1338
55/1338
54/1338
45/1338
62/1337
63/1338
62/1338
64/1337
68/1338
63/1338
70/1337
66/1337
62/1336
54/1338
57/1338
61/1337
61/1336
85/1316
62/1334
63/1336
56/1330
50/1338
79/1338

99.4
91.55

Amycolatopsis palatopharyngis JCM 12460T (AF479268)
51/1338
51/1338
51/1338
47/1338
48/1337
60/1338
58/1338
61/1337
62/1338
63/1337
53/1338
56/1338
52/1338
54/1337
59/1338
56/1338
52/1338
45/1338
60/1337
61/1338
62/1338
64/1337
66/1338
63/1338
67/1337
62/1337
62/1336
56/1338
55/1338
63/1337
64/1336
84/1316
63/1334
64/1336
58/1330
48/1338
80/1338
8/1342

Amycolatopsis fastidiosa DSM 43855T (AB184566)
102/1338
102/1338
102/1338
102/1338
103/1337
128/1339
120/1339
119/1337
123/1338
118/1337
112/1338
112/1338
113/1338
110/1337
119/1338
115/1338
120/1339
110/1339
114/1337
115/1339
115/1338
119/1337
119/1338
116/1338
123/1337
119/1337
115/1336
115/1338
114/1338
122/1338
117/1337
131/1316
116/1336
117/1337
120/1331
109/1338
135/1339
113/1338
110/1338

Exhibit 4

>al-27F

GCTGAAGGACCTTACGGGTGTGGATGAGTGGCGAACGGGTGAGTAACACGTGGGTAACCT
GCCCTGTACTTTGGGATAAGCCCTGGAAACGGGGTCTAATACCGAATATGACTCGCTAAG
GCATCTTGGTGGGTGGAAAGTTTCGGCGGTACAGGATGGGCCCGCGGCCTATCAGCTTGT
TGGTGGGGTAGTGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGTGACCGGCC
ACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCAC
AATGGGCGGAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAA
CCTCTTTCGCCAGGGACGAAGGCGAAGTGACGGTACCTGGAGAAGAAGCACCGGCTAACT
ACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCGAAGCGTTGTCCGGAATTATTGGGCCTA
AAGAGCTCGTAGGCGGCTTGTCGCGTCTGCTGTCAAAATCCGGGGCTTAACTCCGGACCT
GCAGTCGATACGGGCAGGCTTGAGTTCGGTAGGGGAGACTCGAATTCCTGGTGTAGCGGT
GAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGTCTCTGGGCCGATACTG
ACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCG
TAAACGATGGGCGCTAGGTGTGGGCGACTTCCACGTTGTCCGTGCCGCTAGCTAACGCATT
AAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCC

>al-1492R

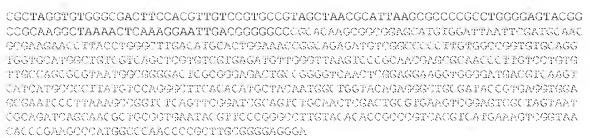
TCCCTCCCCGCAAGCGGGGTTGGGCCATGGGCTTCGGGTGTTACCGACTTTCATGACGTG
ACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCAGCGTTGCTGATCTGCGATTA
CTAGCGACTCCGACTTCACGCAGTCGAGTTGCAGACTGCGATCCGAACTGAGACCGGCTT
TAAGGGATTCGCTCCACCTACGGGTATCGCAGCCCTCTGTACCAGCCATTGTAGCATGTG
TGAAGCCCTGGACATAAGGGGCATGATGACTTGACGTCATCCCCACCTTCCTCCGAGTTG
ACCCCGGCAGTCTCCCGCGAGTCCCCGCCATTACCGCGTGGCAACACAGGACAAGGGCTTG
CGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCACCA
CCTGCACACCGCCCACAAGGGGGCCGACATCTCTCCCGGTTTCCAGTCGATGTCAAGCCC
AGGTAAGGTTCTTCGCGTTGCATCGAATTAATCCACATGCTCCGCCGCTTGTGCGGGCCC
CCGTCAATTCCTTTGAGTTTAGCCTTGCGGCCGTACTCCCCAGGCGGGGCGCTTAATGC
GTTAGCTACGGCACGGACAACGTGGAAGTCGCCCACACCTAGCGGCCCAACGTTTACAGCG
TGGACTACCAGGGTATCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTA
TCGGCCCAGAGACCCGCCTTCGCCACCGGTGTTCCTCCTGATATCTGCGCATTTCACCGC
TACACCAGGAATTCCAGTCTCCCCTACCGAACTCAAGCCTGCCCGTATCCACTGCAGGTC
CGGAGTTAAGCCCCGGAT

>al-1492R Inv compl

ATCCGGGGCTTAACTCCGGACCTGCAGTGGATACGGGCAGGCTTGAGTTCGGTAGGGGAGACTGGAATTCCTGGTG
TAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGTCTCTGGGCCGATACTGACGCTGAGG
AGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGGGCGCTAGGTGTGGGC
GACTTCCACGTTGTCCGTGCCGCTAGCTAACGCATTAAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTC
AAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGATTAATTCGATGCAACGCGAAGAACCTTACCTGG
GCTTGACATGCACTGAAAACCGGCAGAGATGTTCGCCCCCTTGTGGTCGGTGTCACAGGTGGTGCATGGCTGTCGTC
AGCTCGTGTTGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTGTGTTGCCAGCGGTAATGGC
GGGGACTCGCGGGAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTC
CAGGGCTTCACACATGCTACAATGGCTGGTACAGAGGGCTGCGATACCGTGAGGTGGAGCGAATCCCTTAAAGCCG
GTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTG
CGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCATGAAAGTCGGTAACACCCGAAGCCGTACGCC
CAACCCCCTTGTCGGGGAGGGA

Seq A

GCTGAAGGACCTTCGGGTGTGGATGAGTGGCGAACGGGTGAGTAACACGTGGGTAACCTGCCCTGTACTTTGG
GATAAGCCCTGGAAACGGGGTCTAATACCGAATATGACTCGCTAAGGCATCTTGGTGGGTGGAAAGTTTCGGC
GGTACAGGATGGGCCCGCGGCCTATCAGCTTGTTGGTGGGGTAGTGGCCTACCAAGGCGACGACGGGTAGCCG
GCCTGAGAGGGTGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAAT
ATTGCACAATGGGCGAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTT
TCGCCAGGGACGAAGCGCAAGTGACGGTACCTGGAGAAGAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGT
AATACGTAGGGTGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCTGCTG
TGAAAATCCGGGGCTTAACTCCGGACCTGCAGTGGATACGGGCAGGCTTGAGTTCGGTAGGGCAGACTGGAAT
TCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGTCTCTGGGCCGATAC
TGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGTTGGG



>b2-27F

GCTGAAGCACCTTACGGGTGTGGATGAGTGGCGAACGGGTGAGTAACACGTGGGTAACCT
GCCCTGTACTTTGGGATAAGCCCTGGAAACGGGGTCTAATACCGAATATGACTCGCTAAG
GCATCTTGGTGGGTGCAAAGTTTCGGCGGTACAGGATGGCCCCGCGGCCTATCAGCTTGT
TGGTGGGGTAGTGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGTGACCGGCC
ACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCAC
AATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAA
CCTCTTTCGCCAGGGACGAAGCGCAAGTGACGGTACCTGGAGAAGAAGCACCGGCTAACT
ACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTGTCCGGAATTATTGGGCGTA
AAGAGCTCGTAGGCGGCTTGTCCGTCTGCTGTGAAAATCCGGGGCTTAACTCCGGACCT
GCAGTGGATACGGGCAGGCTTGAGTTCGGTAGGGGAGACTGGAATTCCTGGTGTAGCGGT
GAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGGTCTCTGGGCCGATACTG
ACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCG
TAAACGTTGGGCGCTAGGTGTGGGCGACTTCCACGTTGTCCGTGCCGTAGCTAACGCATT
AAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCC
CGCACAAGC

>b2-1492R

CCCTCCCCGCAAGCGGGGTTGGGCCATGGCTTCGGGTGTTACCGACTTTCATGACGTGA
CGGGCGGTGTGTACAAGGCCCGGGGAACGTATTCACCGCAGCGTTGCTGATCTGCGATTAC
TAGCGACTCCGACTTCACGCAGTCGAGTTGCAGACTGCGATCCGAACTGAGACCGGCTTT
AAGGGATTCGCTCCACCTCACGGTATCGCAGCCCTCTGTACCAGCCATTGTAGCATGTGT
GAAGCCCTGGACATAAGGGGCATGATGACTTGACGTCATCCCCACCTTCCTCCGAGTTGA
CCCCGGCAGTCTCCCGCGAGTCCCCGCCATTACGCGCTGGCAACACAGGACAAGGGTTGC
GCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCACCAC
CTGCACACCGGCCACAAGGGGGCCGACATCTCTGCCGGTTTCCAGTGCATGTCAAGCCCA
GGTAAGGTTCTTCGCGTTGCATCGAATTAATCCACATGCTCCGCCGCTTGTGCGGGCCCC
CGTCAATTCCTTTGAGTTTTAGCCTTGCGGCCGTACTCCCCAGGCGGGGCGCTTAATGCG
TTAGCTACGGCACGGACAACGTGGAAGTCGCCCACACCTAGCCCCCAACGTTTACAGCGT
GGACTACCAGGGTATCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTAT
CGGCCCAGAGACCGCCTTCGCCACCGGTGTTCCTCCTGATATCTGCGCATTTCACCGCT
ACACCAGGAATTCCAGTCTCCCCCTACCGAACTCAAGCCTGCCCGTATCCACTGCAGGTC
CGGAGTTAAGCCCCGGAT

>b2-1492R

ATCCGGGGCTTAACTCCGGACCTGCAGTGGATACGGGCAGGCTTGAGTTCGGTAGGGGAGACTGGAATTCCTGGT
GTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGGTCTCTGGGCCGATACTGACGCTGAG
GAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATTGGGCGCTAGGTGTGGG
CGACTTCCACGTTGTCCGTGCCGTAGCTAACGCATTAAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACT
CAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGATTAATTCGATGCAACGCGAAGAACCTTACCTG
GGCTTGACATGCAC TGGAAACGGGCAGAGATGTCGGCCCCCTTGTGGCCGGTGTGCAGGTGGTGCATGGCTGTCGT
CAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAAC GAGCGCAACCCTTGTCCTGTGTTGCCAGCGCGTAATGG
CGGGGACTCGCGGGAGACTGCCGGGGTCAAC TCGGAGGAAGGTGGGGATGACGTCAAGTCATC ATGCCCCTTATGT
CCAGGGCTTCACACATGCTAC AATGGCTGTTACAGAGGGCTGCGATACCGTGAGGTGGAGCGAATCCCTTAAAGCC
GGTCTCAGTTCGGATCGCAGTCTGCAAC TCGACTGCGTGAAGTC GGAGTCGCTAGTAATC GCAGATCAGCAACGCT
GCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCATGAAAGTCGGTAACACCCGAAGCCCATGGC
CCAACCCCAGCTTGCGGGGAGGG

Seq B

GCTGAAGCACCTTCGGGTGTGGATGAGTGGCGAACGGGTGAGTAACACGTGGGTAACCTGCCCTGTACTTTGG
GATAAGCCCTGGAAACGGGGTCTAATACCGAATATGACTCGCTAAGGCATCTTGGTGGGTGGAAAGTTTCGGC
GGTACAGGATGGGCCCGCGGCCTATCAGCTTGTTGGTGGGGTAGTGGCCTACCAAGGCGACGACGGGTAGCCG
GCCTGAGAGGGTGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAAT
ATTGCACAATGGGCGAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTT
TCGCCAGGGACGAAGCGCAAGTGACGGTACCTGGAGAAGAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGT

>c1-27F

NCTGAAGCACCTTCGGGTGTGGATGAGTGGCGAACGGGTGAGTAACACGTGGGTAACCTG
CCCTGTACTTTGGGATAAGCCCTGGAAACGGGGTCTAATACCGAATATGACTCGCTAAGG
CATCTTGGTGGGTGGAAAGTTTCGGCGGTACAGGATGGGCCCGCGGCCTATCAGCTTGTT
GGTGGGGTAGTGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGTGACCGGCCA
CACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACA
ATGGGCGGAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAAC
CTCTTTCGCCAGGGACGAAGCGCAAGTGACGGTACCTGGAGAAGAAGCACCGGCTAACTA
CGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTGTCCGGAATTATTGGGCGTAA
AGAGCTCGTAGGCGGCTTGTCGCGTCTGCTGTGAAAATCCGGGGCTTAACTCCGGACCTG
CAGTGGATACGGGCAGGCTTGAGTTCGGTAGGGGAGACTCGAATTCCTGGTGTAGCGGTG
AAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGGTCTCTGGGCCGATACTGA
CGCTGAGGAGCCAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCTGT
AAACGTTGGGCGCTAGGTGTGGGCGACTTCCACGTTGTCCGTGCCGTAGCTAACGCATTA
AGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCC

>c1-1492R

CCCTCCCCGCAAGCGGGGTTGGGCCATGGGCTTCGGGTGTTACCGACTTTCATGACGTGA
CGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCAGCGTTGCTGATCTGCGATTAC
TAGCGACTCCGACTTCACGCAGTCGAGTTGCAGACTGCGATCCGAACTGAGACCGGCTTT
AAGGGATTCGCTCCACCTCACGGTATCGCAGCCCTCTGTACCAGCCATTGTACCATGTGT
GAAGCCCTGGACATAAGGGGCATGATGACTTGACGTCATCCCCACCTTCCTCCGAGTTGA
CCCCGGCAGTCTCCCGCGAGTCCCCCCCATTACGCCCTGGCAACACAGGACAAGGGTTGC
GCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCACCAC
CTGCACACCGGCCACAAGGGGGCCGACATCTCTGCCGGTTTCCAGTGCATGTCAAGCCCA
GGTAAGGTTCTTCGCGTTGCATCGAATTAATCCACATGCTCCGCCGCTTGTGCGGGCCCC
CGTCAATTCCTTTGAGTTTTAGCCTTGCGGCCGTACTCCCCAGGCGGGGCGCTTAATGCG
TTAGCTACGGCACGGACAACGTGGAAGTCGCCCACACCTAGCGCCCCAACGTTTACAGCGT
GGACTACCAGGGTATCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTAT
CGGCCCAGAGACCCGCCTTCGCCACCGGTGTTCCTCCTGATATCTGCGCATTTCACCGCT
ACACCAGGAATTCCAGTCTCCCCTACCGAACTCAAGCCTGCCCGTATCCACTGCAGGTCC
GGAGTTAAGCCCCGGAT

>c1-1492F

ATCCGGGGCTTAACTCCGGACCTGCAGTGGATACGGGCAGGCTTGAGTTCGGTAGGGGAGACTGGAATTCCTGGTGTAGC
GGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGGTCTCTGGGCCGATACTGACGCTGAGGAGCGAAAG
CGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGTTGGGCGCTAGGTGTGGGCGACTTCCACGTT
GTCCGTGCCGTAGCTAACGCATTAAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGG
GCCCGCACAAGCGGCGGAGCATGTGGATTAATTCGATGCAACGCGAAGAACCTTACCTGGGCTTGACATGCACTGGAAAC
CGGCAGAGATGTCGGCCCCCTTGTGGCCGGTGTGCAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGT
TAAGTCCCGCAACGAGCGCAACCCTTGTCCTGTGTTGCCAGCGCGTAATGGCGGGGACTCGCGGGAGACTGCCGGGGTCA
ACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCTTCACACATGCTACAATGGCTGGTAC
AGAGGGCTGCGATACCGTGAGGTGGAGCGAATCCCTTAAAGCCGGTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCG
TGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCA
CGTCATGAAAGTCGGTAACACCCGAAGCCCATGGCCCAACCCCGCTTGCGGGGAGGG

Seq C

NCTGAAGCACCTTCGGGTGTGGATGAGTGGCGAACGGGTGAGTAACACGTGGGTAACCTGCCCTGTACTTTGG
GATAAGCCCTGGAAACGGGGTCTAATACCGAATATGACTCGCTAAGGCATCTTGGTGGGTGGAAAGTTTCGGC
GGTACAGGATGGGCCCGCGGCCTATCAGCTTGTTGGTGGGGTAGTGGCCTACCAAGGCGACGACGGGTAGCCG
GCCTGAGAGGGTGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAAT
ATTGCACAATGGGCGGAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAAGCTCTT
TCGCCAGGGACGAAGCGCAAGTGACGGTACCTGGAGAAGAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGT
AATACGTAGGGTGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCTGCTG
TGAAAATCCGGGGCTTAACTCCGGACCTGCAGTGGATACGGGCAGGCTTGAGTTCGGTAGGGGAGACTGGAAT

TCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGTCTCTGGGCCGATAC
TGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACCGCTGTAAACGTTGGG
CGCTAGGTGTGGGCGACTTCCACGTTGTCCGTGCCGTAGCTAACGCATTAAGCGCCCGCCTGGGGAGTACGG
CCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGATTAATTCGATGCAACG
CGAAGAACCTTACCTGGGCTTGACATGCACTGGAAACCGGCAGAGATGTCGGCCCCCTTGTGGCCGGTGTGCAGGTGGTG
CATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTGTGTTGCCAGCG
CGTAATGGCGGGGACTCGCGGGAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTT
ATGTCCAGGGCTTCACACATGCTACAATGGCTGGTACAGAGGGCTGCGATACCGTGAGGTGGAGCGAATCCCTTAAAGCC
GGTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGG
TGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCATGAAAGTCGGTAACACCCGAAGCCCATGGCCCAACCCC
GCTTGCGGGGAGGG

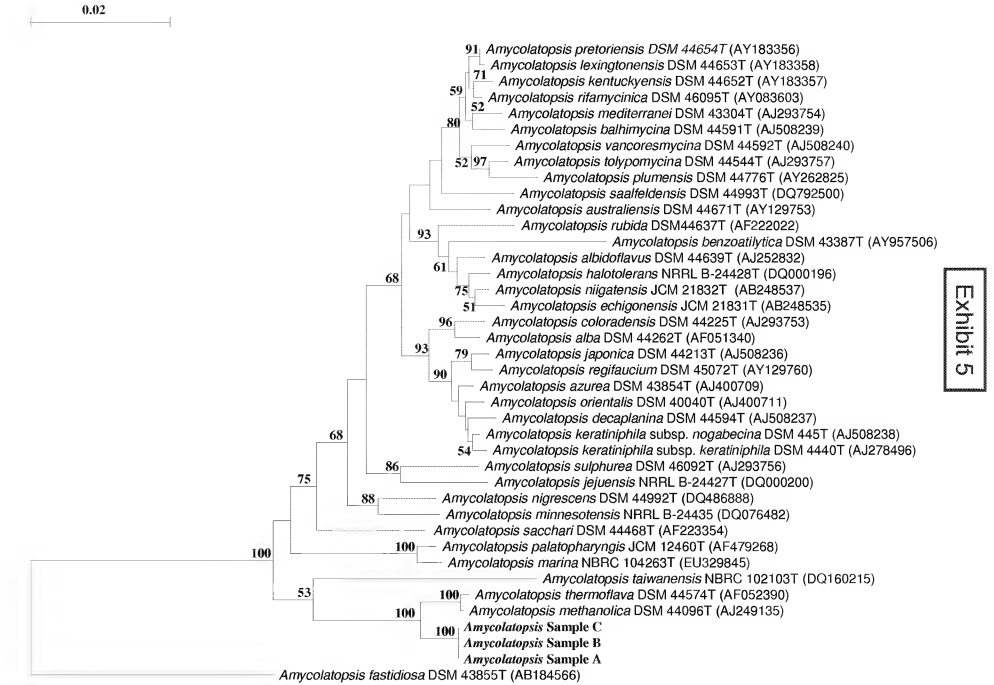

0.02
91 Amycolatopsis pretoriensis DSM 44654T (AY183356)
Amycolatopsis lexingtonensis DSM 44653T (AY183358)
71 Amycolatopsis kentuckyensis DSM 44652T (AY183357)
59 Amycolatopsis rifamycinica DSM 46095T (AY083603)
52 Amycolatopsis mediterranei DSM 43304T (AJ293754)
80 Amycolatopsis balhimycina DSM 44591T (AJ508239)
Amycolatopsis vancoresmycina DSM 44592T (AJ508240)
52 97 Amycolatopsis tolypomycina DSM 44544T (AJ293757)
Amycolatopsis plumensis DSM 44776T (AY262825)
Amycolatopsis saalfeldensis DSM 44993T (DQ792500)
Amycolatopsis australiensis DSM 44671T (AY129753)
Amycolatopsis rubida DSM44637T (AF222022)
93 Amycolatopsis benzoatilytica DSM 43387T (AY957506)
Amycolatopsis albidoflavus DSM 44639T (AJ252832)
61 Amycolatopsis halotolerans NRRL B-24428T (DQ000196)
68 75 Amycolatopsis nigatensis JCM 21832T (AB248537)
51 Amycolatopsis echigonensis JCM 21831T (AB248535)
96 Amycolatopsis coloradensis DSM 44225T (AJ293753)
Amycolatopsis alba DSM 44262T (AF051340)
93 79 Amycolatopsis japonica DSM 44213T (AJ508236)
Amycolatopsis regifaucium DSM 45072T (AY129760)
90 Amycolatopsis azurea DSM 43854T (AJ400709)
Amycolatopsis orientalis DSM 40040T (AJ400711)
Amycolatopsis decaplanina DSM 44594T (AJ508237)
Amycolatopsis keratiniphila subsp. nogabecina DSM 445T (AJ508238)
68 54 Amycolatopsis keratiniphila subsp. keratiniphila DSM 4440T (AJ278496)
86 Amycolatopsis sulphurea DSM 46092T (AJ293756)
75 Amycolatopsis jejuensis NRRL B-24427T (DQ000200)
88 Amycolatopsis nigrescens DSM 44992T (DQ486888)
Amycolatopsis minnesotensis NRRL B-24435 (DQ076482)
Amycolatopsis sacchari DSM 44468T (AF223354)
100 100 Amycolatopsis palatopharyngis JCM 12460T (AF479268)
Amycolatopsis marina NBRC 104263T (EU329845)
Amycolatopsis taiwanensis NBRC 102103T (DQ160215)
53 100 Amycolatopsis thermoflava DSM 44574T (AF052390)
100 Amycolatopsis methanolica DSM 44096T (AJ249135)
100 Amycolatopsis Sample C
Amycolatopsis Sample B
Amycolatopsis Sample A
Amycolatopsis fastidiosa DSM 43855T (AB184566)

Exhibit 5

Exhibit 6

EXTERNAL MEMORANDUM

Re : Report of production trials according to US 6,133,003 to Rabenhorst et al.

Table of contents

Zone Artisanale 40140 Soustons (France)
Tél. : (33) 05.58.41.34.01 Fax : (33) 05.58.41.58.25
http : www.saffias.com
Société Anonyme au capital de 1.830.000 € N° SIRET : 339 013 491 00011 APE : 1039 Z
N° Intracommunautaire : FR 20 339 013 491

1. Goal

The goal is to compare two strains :

- *Amycolatopsis* sp. DSM 9992
- *Amycolatopsis* sp Zyl 926 (referred to also as Asp L-ii 2)

when cultivated in identical conditions, namely identical inoculating chain, fermentation process and equipment.
These two strains were evaluated for the production of vanillin by reducing to practice the process taught in US Patent 6,133,003 to Rabenhorst et al. sometimes referred to as the Symrise process in the following. At the end of the present memo, some results are given while using strain Zyl 926 (Asp L-ii-2) according to the process taught in US Application N° 10/550,945 to Heald et al, referred to as the Salisis process.

2. Material and Methods

2.1 Cultures work out

The process applied in this study was according to the teaching of US Patent 6,133,003 to Rabenhorst et al "Process for the preparation of vanillin and microorganism suitable therefor".

2.1.1 Inoculating chain

- According to working Example 1 of US 6,133,003

Preparation of the preliminary culture

A 500 ml conical flask with a side baffle was filled with 100 ml of medium, comprising 1 g of malt extract, 0,4 g of glucose and 0,4 g of yeast extract and made up to 100 ml with water and then sterilized for 20 min at 121°C. After cooling, the flask was inoculated with 200 µl of a frozen glycerol culture of Amycolatopsis sp. DSM 9992 or Zyl 926 (Asp L-ii 2) The cultures were incubated on a rotary shaking machine at 45 °C and 100 rpm After 24 h, these cultures were used to inoculate the production medium.

- Preliminary culture medium preparation

Product	Concentration
Glucose	4 g/Kg
Malt extract	10 g/Kg
Yeast extract	4 g/Kg
H_2O	q.s.p
pH not adjusted	
Sterilization 20' at 121°C	

- Preculture Inoculation

*Inoculation of 100 mL with 0,2 mL from the content of a cryotube (frozen glycerol culture)

*Incubation (shaking machine) : 45°C - 100 rpm - 24h

2.1.2 Cultures in fermenters (maximum working volume = 15L)

- **Stage production according to working Example 3 of US 6,133,003**

Vanillin Production in a 10 L fermenter.

5 l of culture medium (4 g/l of glucose, 10 g/l of malt extract and 6 g/l of yeast extract) were sterilized in a fermeter and, after cooling, were inoculated with 100 ml of a seed culture of DSM 9992 or Zyl 926 (Asp L-ii 2) according to Example 1.

According to working Example 3, the culture conditions were : 37°C, 500 rpm, 5 l of air/min. 12,5 h after inoculation, 1.634 kg of an approximately 3.7% strength ferulic acid solution (60.2 g of ferulic acid) were added. After 17.5 h, a further 4.397 kg of an approximately 3.7% strength ferulic acid solution (164.72 g of ferulic acid) were pumped in over a period of 10 h.
While trying to reproduce these very same parameters, I had to adapt some of the above data to make it work and arrive at the following parameters :

- Culture parameters

	GROWTH	BIOCONVERSION	BIOCONVERSION
Inoculation	100 mL	-	-
Temperature	37°C	37°C	37°C
Medium quantity	5 Kg	1.6 Kg	4.4 Kg
Culture condition	Batch	Batch (isolated addition of ferulic acid)	**Fed-batch during 10 h (7.2 mL/min)**
Duration	12.5h	5h	14.5h
pO_2 Regulation	50% (not mentioned in the example 3 of the patent)		
Aeration	5 L/min	7 L/min	7 L/min
Stirring	400 à 750 rpm		
pH regulation	8 ± 0.5 (not mentioned in the example 3 of the patent but in the spec.)		
Surpression	No		
End of culture	-	-	32 h

- Fermenters preparation

Product	Concentration
Glucose	**4 g/Kg**
Malt extract	**10 g/Kg**
Yeast extract	**6 g/Kg**
Propylene glycol P2000	**0.35 g/Kg**
H_2O	**q.s.p**
5 Kg of medium in the fermenter *in situ* sterilization	

- Ferulic acid solution

Product	Concentration
Ferulic acid	3.75%
pH adjustment : 7.4 with a sodium hydroxyde solution*	

*preliminary tests to dissolve ferulic acid in water were carried out in vain. I had to add sodium hydroxide otherwise I could not get any homogeneous solution.

Detailed sequence of addition of the ferulic acid solution

- At T12,5 h of culture, 1,6 kg of the ferulic solution were added in the fermenter

- For the semi-continuous step which starts at 17,5 h of culture, 1,3 kg of solution were prepared to feed in the fermenter. It was necessary to reload the solution every 2,5 h because a degradation of ferulic acid is observed when the solution is prepared more than 3 h in advance. The flow rate is 7,2 ml/min during 10 h, allowing to add 4,4 kg of ferulic acid solution.

- After 32 h, the fermentation was terminated.

2.2 Culture follow up

2.2.1 Culture parameters

For each culture, parameters were monitored as follows :

- pO2 (dissolved oxygen in the medium) which allows to visualize the bacterial growth by the more or less important oxygen demand. This parameter is controlled by regulating aeration and/or agitation of the medium to a certain level in order to secure optimal growth conditions
- pH is regulated to favor bacterial growth and the production of metabolites of interest
- Solutions used for pH regulation [sulfuric acid (H_2SO_4) and/or sodium hydroxyde (NaOH)]

2.2.2 Bacteriological follow up

A bacterial follow up was realized in order to be ascertain the absence of any contamination

2.2.3 HPLC Analyses

For each culture, several samples were withdrawn at mentioned times and frozen until analyzed :

T0
T4
T8
T12,5 after spot addition of ferulic acid
T17,5 after the starting of the semi-continuous culture
T20 before reloading of the ferulic acid solution
T22,5 before reloading of the ferulic acid solution
T25 before reloading of the ferulic acid solution
T27,5 before termination of the semi-continuous culture
T32

Production of vanillin, vanillic acid, vanillic alcohol, guaiacol and of the ferulic acid consumption were assayed by HPLC (high pressure liquid chromatography) and UV detection, consumption of glucose was tested through HPLC and refractometry

Two dilutions were made for each sample in order to be sure to be in the detection range of the metabolites.

3. Results

3.1 Culture follow up

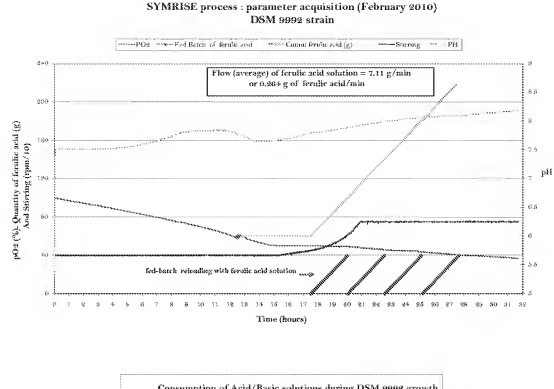
3.1.1 DSM 9992

As illustrated by Figure 1, growth phase went fine, as shown by the pO2 which decreased from 100 % to 57 % between T0 and T12,5 h. This pO2 value had slightly risen during the spot addition of ferulic acid (to 61 %) then decreased again regularly to reach 36 % at the termination of the culture. The 50 % instruction value for pO2 could not be maintained, though agitation was set at a high limit but pO2 did not drop under 30 %, thus oxygen supply has been sufficient throughout the culture duration.

Regarding pH regulation, the addition of acid and/or base solution was not necessary, let alone at the starting of the culture to restore the pH at 8,0 ± 0,5.

At T24 h, I observed a high foam production necessitating the addition of antifoam propylene glycol P2000.

At the termination of the culture, the culture medium was of a yellow/orange color, greasy –probably due to the presence of antifoam) and I noticed very high levels of clove odor/spice, showing *a priori* that bioconversion practically did not occur.



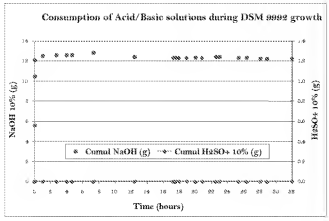
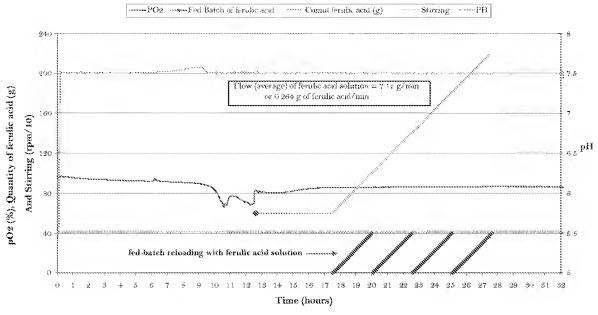


Figure 1 : Parameter acquisition for DSM 9992 strain cultivated according to Symrise process (US 6,133,033) (Cumul = Total)

3.1.2 Zyl 926 (Asp L.ii 2)

As shown in Figure 2, this experiment took place with noticeable differences compared to the previous one with strain DSM 9992 :

- pO2 decreased regularly during growth phase then went over 80 % after the spot addition of ferulic acid
- pH regulation by base solution addition only after addition of ferulic acid during the bioconversion (total amount added : 120g of NaOH 10%).



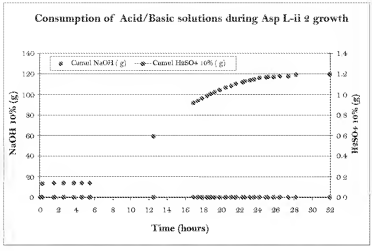


Figure 2 : Parameter acquisition for the Zyl 926 (Asp L-ii 2) strain cultivated according to the Symrise process (US 6,133,033)

3.1.3 Cultures comparison

Table 1 illustrates the principal data :

Table 1 : Growth comparison of DSM 9992 and Zyl 926 (Asp L-ii 2) strains

	pO2	pH	pH regulation with sodium hydroxide 10%	pH regulation with sulfuric acid 10%	Medium composition at the end of the culture
DSM 9992	**Growth phase** (between 0 - 12 h): decrease from 100% to 57% **Bioconversion phase** (between 12 - 32 h) : increase to 61% after ferulic acid addition, then decrease until the end (36% at 32h)	Low initial pH (5.4) → addition of 15g of sodium hydroxide 10% to adjust pH at 8.0 ± 0.5	**Bioconversion phase** (between 12 - 32 h) : NO	**NO**	Oily medium (due to antifoam), yellow/orange, strong smell like a clove
Zyl 926 (Asp L-ii 2)	**Growth phase** (between 0 - 12 h): continuous decrease from 100% to 69% **Bioconversion phase** (between 12 - 32 h) : increase to 85% after ferulic acid addition, then static until the end	No need to adjust initial pH	Continuous addition of sodium hydroxide 10% during bioconversion phase (quantity : 120g)	**NO**	Orange medium, smell like butter

3.2 Analytical results

Table 2 allows to compare the results after the termination of the culture for the two strains :

Table 2 : End product concentrations for Zyl 926 (Asp L-ii 2) and DSM 9992 strains when cultivated according to Example 3 of US 6,133,003

Strain	Vanillic alcohol (g/Kg)	Vanillic acid (g/Kg)	Vanillin (g/Kg)	Guaiacol (g/Kg)
Asp L-ii 2	0	0	0	0
DSM 9992	**<0.1**	**0.3**	**<0.1**	**0**

Chromatograms below clearly indicate that there was an accumulation of ferulic acid over time, whatever strain was used.
No metabolite of interest was detected for strain Zyl 926 (Asp L-ii 2) as illustrated in Figure 3 below, and as shown in Figure 4 below, almost none in the case of strain DSM 9992 : under 0,1 g/kg (0,1 g/l) regarding vanillin, which value should be interpreted as follows : practically no vanillin was produced contrary to the statement in Example 3 of US 6,133,003.

Moreover, at T17,5 h-T20 h-T22,5 h-T25 h and T27,5 h, analysis of intermediary samples was conducted and showed no evidence of the presence of metabolites which could have been produced and then eventually degraded during the cultivation period.

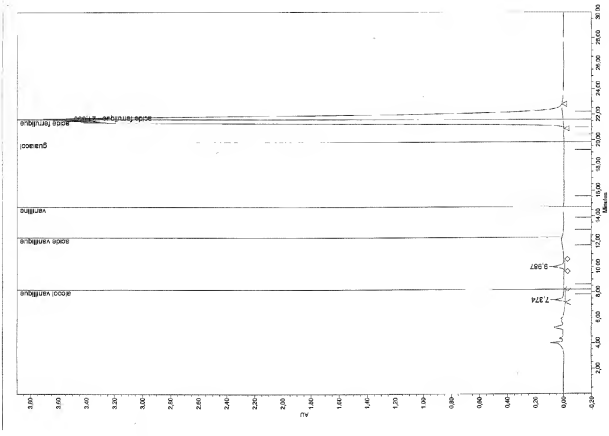


Figure 3 : Chromatogram/Symrise process (US 6,133033) with Zyl 926 strain (Asp L-ii 2) – sample corresponding to the end of the culture (T32 h)
[French/English translation, alcool vanillique/vanillic alcohol – acide vanillique/vanillic acid – vanilline/vanillin – guaiacol/guaiacol – acide férulique/ferulic acid]

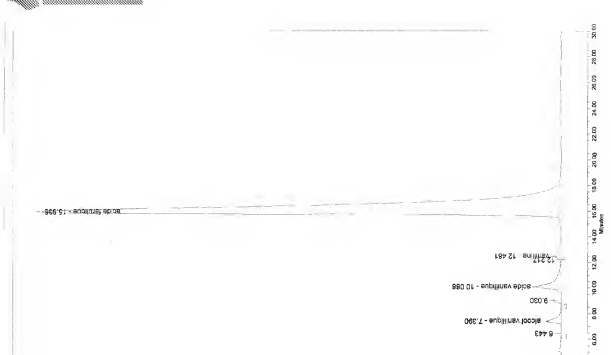


Figure 4 : Chromatogram/Symrise process (US 6,133,033) with DSM 9992 strain – sample corresponding to the end of the culture (T32 h)
[French/English translation: alcool vanillique/vanillic alcohol – acide vanillique/vanillic acid – vanilline/vanillin – guaiacol/guaiacol – acide férulique/ferulic acid]

Table 3 illustrates the accumulation profile of ferulic acid during cultures.

Table 3 : Accumulation profile of ferulic acid in the culture medium

Sample	Ferulic acid Theoretical quantity added (g)	Ferulic acid Theoretical quantity cumulated* (g)	Ferulic acid Theoretical concentration* (g/Kg)	Ferulic acid HPLC results (g/Kg)
T12.5 after addition of ferulic acid solution	60.2 g	-	9.1 g/Kg	8.9 (± 0.3)**
T20 before fed-batch reloading	48.75 g	108.9 g	13.8 g/kg	15.0 (± 2.4)**
T22.5 before fed-batch reloading	48.75 g	157.7 g	17.1 g/Kg	17.3 (± 1.8)**
T25 before fed-batch reloading	48.75 g	206.5 g	19.7 g/Kg	18.5 (± 1.6)**
T27.5 at the end of fed-batch culture	48.75 g	255.3 g	21.6 g/Kg	21.1 (± 0.8)**
T32				19.8 (± 1.1)**

*Two hypothesis :
- no addition of sodium hydroxide to regulate pH
- no consumption and/or loss of ferulic acid between fed-batch reloading
** Result average (g/Kg)

As illustrated by theoretical calculations reported in Table 3 above, bioconversion phase was conducted as taught in US patent 6,133,003 but ferulic acid accumulated and its consumption and degradation after introduction in the culture medium are negligible.

4. Conclusion

Though conducted with identical culture conditions, the above analytical results allow to conclude that strain DSM 9992, claimed in US 6,133,003 and Zyl 926 (Asp L-ii 2), claimed in US Application N° 10/550,945, have shown a different behavior when grown in fermenters. Besides, in both cases growth phase went well.
Moreover, HPLC analysis were performed in order to quantify metabolites of interest, namely vanillin, guaiacol, vanillic alcohol and vanillic acid expected to be produced in such conditions.
These trials results allow us to conclude that no ferulic acid reacted and the latter accumulated in the culture medium during the course of the culture.

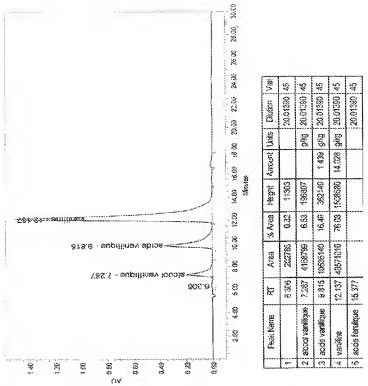
These were unexpected results since the parameters applied in the present study were those taught in working Examples of the document US 6,133,003 to Rabenhorst et al., "Process for the preparation of vanillin and microorganisms suitable therefor".

Moreover we, present engineers who signed the appended affidavits, made all reasonable attempts to use the complete teaching of this specification and not only the Example wording. See for example to name but a few "adjustments", the preparation of ferulic acid and also pH and pO_2 regulation and antifoam necessary usage.

As a conclusion, and based on the forgoing facts, this prior art, namely US 6,133,003 is of a non operative kind, the reason being that a person skill in the art by repeating/reproducing its teaching not only does not get the expected results but rather gets essentially no production of the desired product, namely vanillin. To arrive at this production, one should "invent" another process.

Accordingly, US 6,133,003 is not at all pertinent, and by far, the production of vanillin as taught and claimed by the inventors of US Application N° 10/550,945 is not obviously derivable therefrom.

By way of comparison below is shown in Figure 5, a chromatogram obtained by working out the process taught in US Application N° 10/550,945 to Heald et al, namely the "Safisis process", using Zyl 926 (Asp L-ii 2) : ferulic acid was spent and the vanillin peak is predominant (14 g/l).



	Peak Name	RT	Area	% Area	Height	Amount	Units	Dilution	Vial
1		6.306	282785	0.32	11903			20/01/2010	45
2	alcool vanillique	7.287	6182799	6.53	196807		g/kg	20/01/2010	45
3	acide vanillique	9.215	10826140	16.48	362146	1.439	g/kg	20/01/2010	45
4	vanilline	12.157	45671010	76.03	1525896	14.226	g/kg	20/01/2010	45
5	alcool téréfurique	15.371						20/01/2010	45

Figure 5 : Chromatogram/Safisis process (US App. N° 10/550,945) with Zyl 926 strain (Asp L-ii 2) - sample withdrawn at the end of the culture

[French/English translation: alcool vanillique/vanillic alcohol – acide vanillique/vanillic acid – vanilline/vanillin – guaiacol/guaiacol – acide férulique/ferulic acid]